ISSN 0104-7760

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BIOETHANOL POTENTIAL FROM HIGH DENSITY SHORT ROTATION WOODY CROPS ON MARGINAL LANDS IN CENTRAL CHILE

Keywords:
Bioenergy
Bioethanol
Short rotation crops
Acacia sp.
Eucalyptus spp

ABSTRACT: Cellulosic ethanol is one of the most important biotechnological products to mitigate the consumption of fossil fuels and to increase the use of renewable resources for fuels and chemicals. Short rotation woody crops (SRWC) have been proposed as the most promising raw material for cellulosic ethanol production, as a result of its several advantages over traditional crops. In order to analyze the potential as crops for lignocellulosic bioethanol production in Chile, SRWC were established with the following species: *Acacia melanoxylon*, *Eucalyptus camaldulensis*, *Eucalyptus globulus* and *Eucalyptus nitens*. These crops were established in two contrasting environments and in three plantation densities. The average theoretical ethanol yield at 48 months reached 395.9 $L \cdot t^{-1}$ for *A. melanoxylon*, 348.7 $L \cdot t^{-1}$ for *E. camaldulensis*, and 363.9 L t^{-1} for *E. nitens*. It can be concluded that there are significant differences in polysaccharides yield between species and time. On the other hand, significant differences were found between environments. In conclusion, this study has shown that the choice of SRWC species used as a source of polysaccharides must take into account the percentage content in biomass and, crucially, the species, planting density, harvest cycle and site must be carefully selected to ensure a high biomass yield per unit area.

Histórico:
Recebido 23/11/2016
Aceito 24/03/2017

POTENCIAL DE BIOETANOL DE CULTURAS LENHOSAS CONDUZIDAS EM ALTA DENSIDADE E CURTA ROTAÇÃO EM TERRAS MARGINAIS NO CHILE CENTRAL

Palavras chave:
Bioenergia
Bioetanol
culturas de curta rotação
Acacia sp.
Eucalyptus spp

RESUMO: Etanol celulósico é um dos mais importantes produtos biotecnológicos para reduzir o consumo de combustíveis fósseis e a aumentar a utilização de recursos renováveis para combustíveis e produtos químicos. Culturas lenhosas de curta rotação (CLCR) têm sido propostas como a mais promissora matéria prima para produção de etanol celulósico, devido às suas várias vantagens sobre culturas tradicionais. Para analisar o potencial de culturas para produção de bioetanol lignocelulósicos no Chile, CLCR foram estabelecidos com as seguintes espécies: *Acacia melanoxylon*, *Eucalyptus camaldulensis*, *Eucalyptus globulus* e *Eucalyptus nitens*. Estas culturas foram estabelecidas em dois ambientes contrastantes e em três densidades de plantio. O rendimento teórico médio de etanol em 48 meses atingiu 395,9 $L \cdot t^{-1}$ para A. melanoxylon, 348,7 $L \cdot t^{-1}$ para *E. camaldulensis* e 363,9 $L \cdot t^{-1}$ para *E. nitens*. Foi concluído que existem diferenças significativas no rendimento de polissacarídeos entre as espécies e o tempo. Por outro lado, foram encontradas diferenças significativas entre ambientes. Em conclusão, este estudo mostrou que a escolha da espécie usada na CLCR como fonte de polissacarídeos deve levar em conta sua percentagem na biomassa e, crucialmente, a espécie, densidade de plantio, ciclo de colheita e sítio devem ser cuidadosamente selecionados para garantir um rendimento elevado de biomassa por unidade de área.

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DOI:
10.1590/01047760201723012278

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INTRODUCTION

Bioethanol is a potentially renewable and environmentally sustainable biofuel. Increasing amounts of bioethanol are being produced by fermentation of sugars derived from mainly sugarcane and cornstarch. Bioethanol from second generation are made from lignocellulosic biomass or woody crops, agricultural residues or waste, non-competitive to food and feed.

The availability of fossil fuels is expected to decline in a near future. Fossil fuels correspond to a non-renewable source that poses environmental concerns and it is subject to price instability (NARAYAN; NARAYAN, 2014). There is clear scientific evidence that the emissions of greenhouse gases (GHG) such as carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) produced by the combustion of fossil fuels and the land use change as a result of the human activity are disrupting the global climate (KANG et al., 2014; SINDHU et al., 2016).

Therefore, new renewable energy sources and chemicals friendly to the environment are the subject of research and development. Logically, the substitution options of raw materials for the petrochemical industry must be based on non-fossil resources and potentially, alleviate the problem of instable prices (NARAYAN; NARAYAN, 2014) and reduce the impact on the global climate (KANG et al., 2014; MORAES et al., 2017). Renewable energy resources have a more uniform distribution than fossil or nuclear resources. In addition, renewables account for 40% of the growth in power generation (BP, 2017). There are several reasons for biofuels to be considered as relevant energies in both industrialized and developing countries. These include energy safety reasons, environmental concerns, currency savings and socioeconomic problems related to rural areas of all countries (DEMIRBAS, 2008; DINCER, 2008).

Biomass and biofuels have been identified by the U.S. Department of Energy as critical technologies in order to minimize the costs of reducing carbon emissions. Co-combustion in coal-fired power plants, integrated gasification in combined cycle units for the forest industry and ethanol coming from hydrolysis of lignocelluloses biomass (LCB) have the greatest potential, with estimated annual carbon offsets in U.S. only ranging between 16 and 24 tons to 2010 (MIEDEMA et al., 2017).

The production of raw material from woody crops in order to produce LCB that generates bioethanol by means of a process of hydrolysis and fermentation to be used as fuel is one of the most promising alternatives. Bioethanol is the most widely used biofuel in the world because of its favorable environmental characteristics (URBANCHUK, 2001). Bioethanol can be obtained from any biological raw material that contains significant amount of sugar or materials that can be converted into sugar, such as starch or cellulose. It is produced from fermentation of sugars present in the LCB through natural or genetically enhanced microorganisms such as yeast and bacteria (EIA, 2013). For the conversion of LCB (in this case short rotation woody crop - SRWC biomass) into bioethanol is necessary to define fractionation methods to obtain fermentable sugars. Different types of pretreatment can modify the LCB in different ways, thereby affecting the accessibility of the materials. Pretreatment can be classified into physical, chemical, physicochemical biological and combined pretreatments (TAHERZADEH; KARIMI, 2008). Autohydrolysis and steam explosion pretreatments are attractive for hardwoods because they can be used without the addition of chemical reagents to destructuralize biomass (autocatalysis) (REYES et al., 2015). Enzymatic hydrolysis of pretreated materials is used for the production of fermentable sugars, which can be converted into biobased fuels and chemicals. LCB pretreatments disrupt and partially open-up the lignocellulose structure by removing lignin and hemicelluloses. This action is required to expose cellulose to increase its saccharification during the enzymatic hydrolysis process (MOSIER et al., 2005).

As biofuel, bioethanol is used in internal combustion vehicles in pure form or as an additive to gasoline (WHITE; PLASKETT, 1981) called Gasohol. Thus, this fuel has the advantage that no modifications in the engine are required to its use, though some modifications are required in higher blends (LAUNDER, 2001).

With the use of E10 greenhouse gas emissions are reduced between 12% and 19% compared to conventional gasoline. In addition, the high oxygen content, combustion is cleaner and is able to reduce by 13% the toxic contents of gasoline and releases 30% less CO_2 than a new vehicle (MORALES et al., 2016). It is highly soluble in water and biodegradable. Ethanol is a renewable fuel produced from plants, unlike petroleum-based fossil fuels, which have a limited supply. Fossil fuels are also the most significant contributor of carbon dioxide into the atmosphere (RFA, 2008). These environmental benefits have increased the interest in using this biofuel. As a result, countries such as Brazil and the US have energy policies on its use and production and becoming the greatest bioethanol producers in the world. This biofuel is produced from sugarcane and corn, respectively. Both countries produce more than 83% of the total bioethanol produced in the World (RFA, 2013).

In Chile, bioethanol is under investigation and the knowledge on the chemical composition of the

lignocellulosic biomass (LCB) is fundamental in order to find optimum raw material, as well as the proper technology for its production (MANSILLA et al., 1991). In this sense, the selection of the woody species is an important issue, because its chemical composition, as well as its physical properties and the features of its growth (FAO, 2001), will affect on its bioethanol production capacity. Species that are intended for bioethanol production, besides its rapid growth and being managed on SRWC, it is desirable they have the capacity of regenerate vegetatively, thus giving rise to successive coppice rotations.

Plantations of *Eucalyptus* spp. intended for bioenergy have been established mostly in Australia, New Zealand, China, South America, the Mediterranean, and Africa (NIEMISTÖ, 1995; MISRA et al., 1998; SOCHACKI et al., 2007). Species of this genus are characterized by rapid growth and high biomass yield, making them the first choice for wood production (MACFARLANE et al., 2004; PARSONS et al., 2004; FORREST; MOORE, 2008). Many studies inform the biomass yield of these species as SRWC's established at diverse stockings (BARTON; MONTAGU, 2006; SOCHACKI et al., 2007; FORREST; MOORE, 2008). In general, these studies agreed that higher stand density higher stand biomass. Furthermore, these researches reported that the biomass yield is maximized among initial stocking 2,500 and 5,000 trees per hectare within rotation of among 3 to 6 year.

Sandoval et al., (2017) recorded in the pioneer dendroenergy crops established in degraded soils of central Chile, species of *Eucalyptus* reached the higher biomass yield and significantly exceeded that of *A. melanoxylon*. Reported in clay rainfed site, *E. camaldulensis* reached the highest total biomass yield with 14.9, 22.5 and 20.3 Mg·ha^{-1} at initial stocking of 5000, 7500 and 10000 trees·ha^{-1}, respectively; meanwhile in sandy site, *E. nitens* had the highest total biomass yield with 23.4, 35.2, and 29.2 Mg·ha^{-1}, respectively.

The aim of this research is to determine the chemical composition of *Acacia melanoxylon*, *Eucalyptus camaldulensis*, *Eucalyptus globulus* and *Eucalyptus nitens* species, established in two sites, under three plantation densities and samples in four consecutive seasons. Finally, their potential as crops for lignocellulosic bioethanol production in Chile will also be analyzed with determination of ethanol yields.

MATERIAL AND METHODS

Collection of biomass samples

The trials were established in August 2007, on the Llohué and Santa Rosa sites, located in the interior dryland of Biobío Region, Chile. Both sites present serious nutritional and hydric deficits, leading to low yields of wood production. The soils of Llohué, located near Ninhue township, are granitic in origin and belong to the Cauquenes series. This land is very susceptible to erosion and is severely compacted. The surface horizons have little organic matter and high gravel contents, which negatively affect the establishment and initial growth of tree plantations. The site is extremely arid, with annual precipitation of 700 mm and up to five months of drought (CARRASCO et al., 1993; DGAC, 2015). Temperatures fluctuate between 0 and 30°C. The topography is undulating and abrupt. The slope of the land in the study area does not exceed 5%. The soils of Santa Rosa site, located near Yungay township, are andesitic basalt in origin and belong to the Arenales series. The topography of this site is flat. Temperatures fluctuate between 0 and 30°C, but can reach 70°C at the surface in summer due to the dark color of the soil. Drainage is excessively high, and the entire profile has important organic matter limitations. Annual precipitation is 1,100 mm, but extreme aridity in summer substantially affects the survival of recently established plantations.

The trial was established as a complete randomized block design (CRBD) with three replicates. Blocks were square of 75 m at each side (5,625 m^2) consisting of nine experimental units of 25 m per side (625 m^2) with 49 measurements tree and a buffer zone to reduce edge effect. At Llohué, three species (*A. melanoxylon*, *E. camaldulensis*, *E. nitens*) were established in each block at three initial stockings (5,000, 7,500, 10,000 trees·ha^{-1}). At Santa Rosa, the trial consisted of a CRBD in split-plot with three species (*A. melanoxylon*, *E. camaldulensis*, *E. globulus*) established at the same three stockings. For *E. camaldulensis* and *E. nitens*, each sub-plot was made up of a mitigation zone of edge effect and a core plot with 45 measurements trees (five rows of nine trees each), whereas for *A. melanoxylon*, the core plot consisted of 15, 24, and 30 trees established at 5,000, 7,500, and 1,0000 trees·ha^{-1}, respectively; the split-plot were to analyze coppice after annual harvests starting from the second year. Four months after establishing this trial, as the number of *A. melanoxylon* trees declined heavily, these units were replanted and stand densities returned to the nominal stocking at the eleventh month.

Measurement of variables and biomass determination per tree and surface unit

Individual tree measurements at each experimental unit were made in October and December 2007, July and December 2008, 2009 and 2010, and July 2011. At each measurements time collar diameter (*D*) at 0.1 m above the ground, diameter at breast height (*DBH*) once the trees where taller than 1.3 m, crow diameter and total height of the all trees were measured for each core experimental unit. For the third, fifth, seventh and ninth measurements (*i.e*. July 2008, 2009, 2010, 2011), above biomass was determined using destructive samples taken from three trees in the edge effect mitigation zone of each experimental unit. The collected trees covered both the diametric (*D*) and total height distribution. These trees were cut at 0.1 m above ground level, transported, and stored at 4 °C. The fresh material was dried at 65 °C to a constant dry weight. The total dry biomass per tree was determined by weighing each component (*i.e*. stem, branches and foliage) separately.

Data from the biomass sampling was used to adjust the relationship $\ln y = b_0 + b_1 \ln (D^2 H)$ at tree level, corresponding to the logarithmic transformation of the model $y = \beta_0 (D^2 H)^{\beta_1}$ which intended to correct heteroscedasticity (BARTON; MONTAGU, 2006). In those models, y is the total aerial biomass of the tree or one of its components (g), D is the collar diameter (mm), and H is the total height of the tree (cm); b_1 and b_2 are regression parameters. As the dependent variable of the model was expressed in logarithmic terms, it was not possible to consider additivity restrictions of tree biomass components (PARRESOL, 2001). Total and component biomass functions were fitted per species and initial stockings; an average fit was also done by species. The fitted functions were used to estimate the total and by component biomass in each experimental unit using as predictors both the collar diameter and total height of each tree in the core plot. This procedure was carried out during each measurement, except the first (*i.e*. October 2007), when biomass was determined as the average of 10 plants per species, considering only the biomass of the tree, timber, and foliage. Total and component biomass per area unit were obtained by extrapolating the estimated biomass of the trees in the useable experimental area unit to the nominal initial stocking.

Chemical characterization of the species

The bark-free wood was splinter to particulate size between 3.0×2.5×0.3 cm in a chipper. Wood chips were milled in a knife mill and sieved through a 45/60 mesh.

Determination of extractable compounds: firstly the humidity content was determined to sawdust samples in a thermobalance, according to the 264 cm-97 Tappi standards. Subsequently, samples of approximately 2 g of dry wood were weighted in a tared extraction thimble. Each extraction thimble was placed on a soxhlet apparatus. 150 mL of solvent in a 250 mL round bottom flask were used. The solvents used were 95% (v/v) ethanol solution followed by 100% acetone. The flask was connected first with ethanol solvent to the soxhlet apparatus and six refluxes per hour during 5 h were performed. After the solvent was changed for acetone and the cycles were repeated. Thimbles were washed with water and allowed to dry in order to eliminate the solvents. Then, samples were oven dried at 105°C. The percentage of extractables was determined gravimetrically.

Determination of both carbohydrates and lignin from lignocellulosic material was carried out by means of a hydrolysis with sulfuric acid using lignin-extracted wood. Samples of lignocellulosic material were treated with a solution of 72% sulfuric acid for 1 h at 30°C in a first stage. Then, samples were treated with a solution of the same acid, for one hour at 120°C (PULS et al., 1985; BAEZA; FREER, 2001). By filtration, sugars were separated and analyzed by means of high performance liquid chromatography (HPLC) (LaChrom-Merck-Hitachi (Tokyo, Japan)). Separation was carried out in a Aminex HPX-87H o la HPX-87P column (300×7.6 mm; BioRad, Hercules, CA), using a refraction index detector at 45°C, a mobile phase of 5×10^{-3} mol·L^{-1} H_2SO_4 and a flow rate of 0.6 mL·min^{-1}. Glucose and xylose were used as external calibration standards. The glucans content was calculated by multiplying the glucose content by 0.9; the xylans content obtained from the xylose content multiplied by 0.88; and the acetyl groups content was calculated by multiplying the acetic acid content by 0.70 according to Elissetche et al., (2006). The solid residue (lignin insoluble in acid) was oven dried at 105°C until constant weight. Then, the lignin content was gravimetrically quantified. All analysis was done in triplicate.

Pretreatments

Autohydrolysis of *E. globulus* SRWC biomass from sandy areas was performed in a 5 gal Parr reactor (Parr Instruments, Moline, IL) loaded with 2000 g of wood chips (dry basis) and water-wood ratio of 6:1 (v/w). The reactor was heated at 3°C·min^{-1}, and the cooking was conducted at 194°C and residence time of 1 min, according to previous work (TRONCOSO et al., 2017).

After autohydrolysis, the pulp was separated from liquor by vacuum filtration. Liquor and pulp were stored at 4°C until its use.

Enzymatic hydrolysis (EH)

For enzymatic hydrolysis, cellulase NS-22128 (71 FPU mL^{-1}) supplemented with β-glucosidase NS-22118 (370 CBU/mL) provided by Novozymes (Denmark) was used. The enzyme dosage utilized was 20 FPU and 20 CBU per gram of pretreated material. Enzymatic hydrolysis was performed in 125 mL Erlenmeyer flasks at substrate concentration of 10% (w/v) in 50 mL sodium citrate buffer (pH 4.8, 0.05 mol·L^{-1}) in an orbital shaking incubator at 50°C and 150 rpm for 72 h. The glucose content released was analyzed by HPLC. The yield is expressed as the percentage of glucose released in the enzymatic hydrolysis divided by the potential glucose available in the pretreated material. All measurements were performed in triplicate.

Simultaneous saccharification and fermentation (SSF)

The yeast strain utilized in this work was a thermal acclimatized (40°C) *Saccharomyces cerevisiae* IR2-9a (ARAQUE et al., 2008). The inoculum was grown in 100 mL of liquid culture made out of glucose, 50 g·L^{-1}; yeast extract, 5 g·L^{-1}; peptone, 5 g·L^{-1}; KH_2PO_4, 1.0 g·L^{-1}; $MgSO_4 7H_2O$, 0.50 g·L^{-1}; NH_4Cl, 2 g·L^{-1} in a 500 mL Erlenmeyer flask. The culture was incubated for 48 h at 40°C in an orbital shaker at 150 rpm.

The simultaneous saccharification and fermentation (SSF) was performed at 10% substrate consistency. In a 125 mL Erlenmeyer flask, the pretreated material with 70% humidity (3 g dry weight) was suspended in a total reaction volume of 30 mL 0.05M citrate buffer solution (pH 4.8). Nutrients, consisting in KH_2PO_4, 1.0 g·L^{-1}; $MgSO_4 7H_2O$, 0.50 g·L^{-1}; peptone, 5.0 g·L^{-1}; yeast extract, 5.0 g·L^{-1}, were added. The SSF of the samples was performed using the same concentration and enzyme preparation used in enzymatic hydrolysis. After enzyme addition, the yeast inoculum was added at 6.0 g· L^{-1} (3.5×10^8-3.3×10^9 yeast cell mL^{-1}). SSF was performed at 40°C and 150 rpm for 96 h. Samples were taken at 24, 48, 72 and 96 h and analyzed for ethanol content by gas chromatography (GC) on a Perkin-Elmer autosystem XL Headspace using a FID detector and a column HPS MS30m column. The GC program was: 50°C×3 min; 10°C·min^{-1}, 100°C×1 min; 25°C min^{-1}, 125°C×1 min. The temperature of the injector and detector were 200 and 300°C, respectively. Ethanol yields were calculated as a percentage of the theoretical yield. The theoretical yield was calculated by dividing the ethanol amount obtained (g) by the amount of glucose in pretreated material (g), assuming that all the potential glucose in the pretreated material is available for fermentation, with a fermentation yield of 0.51 g ethanol g $glucose^{-1}$ multiplied by 100. All the determinations were performed in triplicate.

Estimating the amount of ethanol

The estimation of the amount of ethanol obtained from wood plantations was calculated by means of the theoretical ethanol production (L·ha^{-1}) in different environments for each age of the crop. By using the individual carbohydrate content (glucane and xylan) obtained from the analysis of the chemical composition, theoretical ethanol yields were calculated in L·t^{-1} using the theoretical ethanol conversion factors by the US Energy Department (DOE, 2013). The calculation was performed based on the following formula [1]:

$$\text{Theoretical ethanol yield } (L\cdot Mg^{-1}) = \left(\%\ \text{C6 sugar} \cdot \frac{1.10\text{kg C6 sugar}}{\text{kg polymeric sugar}} \cdot \frac{0.51\text{kg ethanol}}{\text{kg sugar}} \cdot \frac{1000\text{kg}}{\text{ton C6 polimeric sugar}} \cdot \frac{1.27\text{L ethanol}}{\text{kg ethanol}}\right) + \left(\%\ \text{C5 sugar} \cdot \frac{1.136\text{kg C5 sugar}}{\text{kg polymeric sugar}} \cdot \frac{0.51\text{kg ethanol}}{\text{kg sugar}} \cdot \frac{1000\text{kg}}{\text{ton C5 polimeric sugar}} \cdot \frac{1.27\text{L ethanol}}{\text{kg ethanol}}\right) \quad [1]$$

In order to assess the theoretical bioethanol yields per hectare of plantations, regarding different densities, species and environment, the following items were calculated: yield of debarked stem wood per hectare and percentage of bioethanol that could be obtained from their sugars content with 90% in the conversion efficiency. With this information it was possible to obtain the amount of bioethanol in liter per wood ton and liters of bioethanol per hectare. Thus, the theoretical bioethanol yield on the basis of a surface unit (L·ha^{-1}) was obtained by multiplying the theoretical bioethanol yield on a biomass basis (L·t^{-1}), as well as the biomass yield (dry matter) in t·ha^{-1}, considering the variables of site, time and species.

Statistical analyses

SAS 9.2 Statistic package (SAS Institute Inc., Cary, NC) was used to carry out all statistical analyses. A Variance analysis (ANOVA) of the repeated measurements was performed through the Proc Mixed module by SAS in order to test the effect of the season, intensity and species on the bioethanol content which was re-sampled in the same place during three consecutive seasons. In all cases the assumptions of normality, constant variance and independence form errors were met. An alpha of 0.05 was used to determine significant differences between seasons or with seasons and densities.

RESULTS

Pretreatment, Enzymatic hydrolysis and SSF from *E. globulus* SRWC biomass

The pretreatment conditions used in this work were determined in a previous optimization work developed in the Bioenercel Consortium framework (TRONCOSO et al., 2017).

The chemical composition of *E. globulus* and the pretreated biomass by autohydrolysis used in this stage are shown in Table 1. The average solid yield obtained after pretreatments was 71.1 ± 2.7% w/w. In the three experiments done was possible to obtain a pretreated biomass with 97% of the cellulose present in *E. globulus* SRWC. The hemicelluloses removed during pretreatment were 81% and in the case of lignin only 4% was removed; both were calculated in dry wood basis. In Figure 1 it is possible to observe the evolution of enzymatic hydrolysis of pretreated biomass in the time, the highest glucose concentration (82.3 ± 1.6% w/w) was detected at 48 h in potential glucose basis present in the raw material. On the other hand, the highest ethanol yield by SSF was 86.9 ± 1.6% w/w at 72 h, in potential ethanol basis that could be produced using the total cellulose present in the raw material.

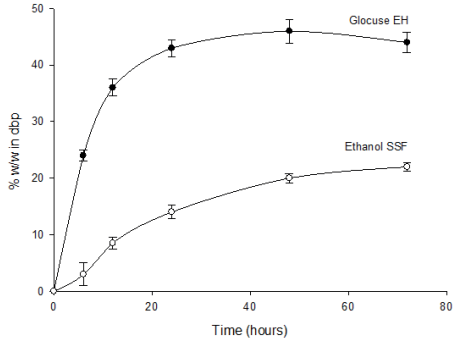

FIGURE 1 Experimental glucose obtained by enzymatic hydrolysis from *E. globulus* SRWC biomass pretreated by autohydrolysis and bioethanol from the same pretreated biomass by simultaneous saccharification and fermentation, both expressed as percentages in dry pretreated biomass base (% w/w dpb).

The ethanol yield for *E. globulus* from SRWC pretreated by autohydrolysis was near to 90% in dry wood basis; this value was used to calculate the theoretical ethanol production to the others hard wood analyzed (Table 2 and 3), because the chemical composition, mainly the presence of acetyls groups in hemicelluloses are similar and responsible to catalyses the autohydrolysis pretreatment (CASTRO et al., 2013).

Variability in content of lignin, glucan, polyoses and acetyl

The individual content of lignin, glucan, polyoses and acetyl are presented in Tables 2 and 3. The data was obtained from the analysis of the composition of the biomass samples harvested after 11, 23, 36 and 48 months in the sites of Llohué and Santa Rosa. The glucan ratio to total carbohydrates for the biomass samples from Llohué was 67.9 ± 2.3% for *A. melanoxylon*, 68.5 ± 1.4% for *E. camaldulensis* and 62.6 ± 1.9% for *E. nitens*. In the case of the samples from Santa Rosa, the content was as follows: 66.2 ± 1.3% for *A. melanoxylon*, 63.9 ± 1.1% for *E. globulus* and 62.7 ± 1.2% for *E. nitens*.

The glucan content of the samples taken from Llohué and Santa Rosa were significantly different over time ($p<0.05$) for the species, as well as for the interaction between both factors (environment – species). The situation is the opposite for the different densities used in each of the environments (Table 4). The significant interaction between the established species and time ($p<0.05$) indicates that the differences in the glucan content between species were not consistent between different measuring times. Most of the variability in the chemical composition was based on time (19.6%, 62.5% and 10.9%), in species (27.0%, 7.3% and 17.4%), and in the interaction between time of measurement and species with only 6.1%, 6.6% and 33.5%.

TABLE 1 Chemical composition of raw material, *E. globulus* SRWC biomass, and pretreated autohydrolysis biomass expressed in dry basis. Solids, glucose by enzymatic hydrolysis and ethanol yields by simultaneous saccharification and fermentation are shown.

Biomass	% dry basis						% dry wood basis		
	Glucans	Hemicelluloses	Acethyl	Lignin	Acetone-ethanol extractives	Ash	Solid yield	Glucose Yield EH 48 h	Ethanol Yield SSF 72 h
E. globulus	39.8±0.3	16.5±0.5	3.7±0.2	25.5±0.06	1.9±0,05	2.0±0.0	-	-	-
E. globulus[1]	54.0±0.3	3.7±0.0	-	35.5±0.3	-	-	71.1	80.8±0.4	88.1±0.4
E. globulus[2]	54.1±0.5	5.1±0.0	-	32.4±0.2	-	-	73.8	82.4±0.6	87.5±0.6
E. globulus[3]	54.1±0.3	4.4±0.1	-	35.6±0.3	-	-	68.4	83.9±0.4	85.1±0.4

[1]-pretreated; [2]- pretreated; [3]-pretreated

TABLE 2 Content of extractable, lignin, glucan, polioses, acetyl, and percentage of theoretical bioethanol of the biomass of species planted in Llohué (rainfed) from 2007 to 2011, calculated using 90% of efficiency in sugars to bioethanol conversion. Values represent the mean and the standard deviation of the sample.

Month	Species	Density	Percentage					% p/p theoretical bioethanol 90% efficiency
			Extractable	Total lignin	Glucan	Polyoses*	Acetyl	
11	*A. melanoxylon*	5,000	5.88 ±0.89	26.35 ±0.60	41.75 ±2.30	15.90 ±2.40	3.80 ±0.00	21.29 ±1.30
11	*A. melanoxylon*	7,500	6.70 ±0.49	27.65 ±0.83	39.87 ±0.99	15.47 ±3.00	3.50 ±3.23	20.33 ±0.56
11	*A. melanoxylon*	10,000	6.09 ±0.16	25.97 ±1.03	38.73 ±4.33	15.07 ±2.77	2.07 ±5.67	19.75 ±2.46
11	*E. camaldulensis*	5,000	5.62 ±0.55	26.95 ±0.47	36.40 ±3.50	17.13 ±1.73	3.80 ±4.37	18.56 ±1.98
11	*E. camaldulensis*	7,500	4.52 ±0.24	28.03 ±0.52	36.87 ±2.12	13.77 ±3.20	3.13 ±2.63	18.80 ±1.20
11	*E. camaldulensis*	10,000	5.12 ±0.41	26.90 ±0.63	38.70 ±1.27	16.37 ±7.20	2.00 ±3.40	19.74 ±0.72
11	*E. nitens*	5,000	7.87 ±0.62	26.60 ±0.20	34.60 ±3.70	18.43 ±2.57	3.53 ±4.30	17.65 ±2.10
11	*E. nitens*	7,500	7.95 ±0.65	26.95 ±0.67	30.73 ±3.43	17.63 ±5.63	2.47 ±0.73	15.67 ±1.95
11	*E. nitens*	10,000	7.71 ±0.64	26.60 ±0.45	32.90 ±2.35	18.90 ±3.55	4.35 ±3.95	16.78 ±1.33
23	*A. melanoxylon*	5,000	4.15 ±0.64	21.43 ±2.63	38.20 ±6.33	22.80 ±6.43	3.47 ±4.63	19.48 ±3.59
23	*A. melanoxylon*	7,500	4.10 ±0.44	21.37 ±2.00	39.40 ±2.60	24.33 ±3.43	3.50 ±2.90	20.09 ±1.47
23	*A. melanoxylon*	10,000	4.55 ±0.35	22.07 ±1.33	38.70 ±1.07	23.23 ±0.97	3.47 ±2.07	19.74 ±0.60
23	*E. camaldulensis*	5,000	4.25 ±0.21	26.07 ±1.77	37.50 ±1.10	23.13 ±1.70	3.10 ±1.63	19.13 ±0.62
23	*E. camaldulensis*	7,500	5.03 ±0.50	25.70 ±2.37	40.00 ±1.23	23.27 ±1.23	3.20 ±1.47	20.40 ±0.70
23	*E. camaldulensis*	10,000	4.10 ±0.14	26.43 ±1.40	38.80 ±0.67	25.43 ±0.80	3.30 ±0.83	19.79 ±0.38
23	*E. nitens*	5,000	3.27 ±0.50	26.00 ±1.73	37.80 ±0.57	28.83 ±2.20	3.97 ±0.83	19.28 ±0.32
23	*E. nitens*	7,500	2.25 ±0.21	25.70 ±2.43	36.87 ±1.67	29.30 ±1.33	4.20 ±2.10	18.80 ±0.94
23	*E. nitens*	10,000	2.90 ±0.57	25.93 ±2.37	36.90 ±1.30	29.57 ±6.87	4.00 ±2.53	18.82 ±0.74
36	*A. melanoxylon*	5,000	5.30 ±0.71	22.33 ±1.90	39.67 ±1.10	18.87 ±4.28	3.53 ±4.83	20.23 ±0.62
36	*A. melanoxylon*	7,500	5.30 ±0.57	20.73 ±1.63	42.30 ±1.60	17.40 ±4.70	3.33 ±3.90	21.57 ±0.91
36	*A. melanoxylon*	10,000	5.84 ±0.83	21.73 ±0.83	39.57 ±1.20	18.67 ±6.45	3.47 ±4.87	20.18 ±0.68
36	*E. camaldulensis*	5,000	6.55 ±0.92	27.03 ±2.50	38.87 ±0.70	15.57 ±4.47	4.17 ±2.03	19.82 ±0.40
36	*E. camaldulensis*	7,500	6.00 ±0.53	28.20 ±0.77	37.77 ±0.90	15.57 ±5.27	3.03 ±2.13	19.26 ±0.51
36	*E. camaldulensis*	10,000	6.20 ±0.85	26.80 ±2.60	38.30 ±0.27	15.50 ±2.90	3.53 ±0.70	19.53 ±0.15
36	*E. nitens*	5,000	7.00 ±0.36	26.53 ±1.40	37.83 ±1.17	20.17 ±5.92	5.30 ±5.17	19.30 ±0.66
36	*E. nitens*	7,500	4.57 ±0.47	28.27 ±0.47	36.37 ±0.60	19.60 ±3.92	4.93 ±1.53	18.55 ±0.34
36	*E. nitens*	10,000	5.65 ±0.84	25.87 ±1.43	35.50 ±3.03	21.03 ±4.20	5.03 ±5.13	18.11 ±1.72
48	*A. melanoxylon*	5,000	5.65 ±0.36	23.62 ±1.13	41.35 ±0.47	19.16 ±1.01	3.93 ±1.35	21.09 ±0.27
48	*A. melanoxylon*	7,500	5.78 ±0.68	23.74 ±3.24	44.04 ±3.80	19.32 ±2.37	3.72 ±2.94	22.46 ±2.16
48	*A. melanoxylon*	10,000	5.98 ±0.79	20.88 ±1.13	42.84 ±1.44	17.60 ±1.85	3.50 ±2.11	21.85 ±0.82
48	*E. camaldulensis*	5,000	6.76 ±0.83	31.57 ±1.35	41.90 ±1.35	15.74 ±2.85	3.36 ±5.37	21.37 ±0.76
48	*E. camaldulensis*	7,500	6.45 ±0.73	28.78 ±1.98	42.68 ±1.32	15.51 ±3.74	3.13 ±7.70	21.77 ±0.75
48	*E. camaldulensis*	10,000	6.15 ±0.75	30.36 ±1.43	37.16 ±2.06	16.73 ±2.88	3.58 ±4.51	18.95 ±1.17
48	*E. nitens*	5,000	6.33 ±0.66	26.76 ±0.96	40.78 ±2.64	19.60 ±3.45	4.43 ±4.92	20.80 ±1.50
48	*E. nitens*	7,500	5.74 ±0.46	29.39 ±1.23	38.43 ±0.82	18.36 ±2.04	2.85 ±6.14	19.60 ±0.47
48	*E. nitens*	10,000	5.78 ±0.56	27.52 ±1.38	37.79 ±2.74	19.38 ±2.81	4.45 ±5.00	19.27 ±1.55

* Represent the concentration of total polyoses from hemiceluloses (xylan, arabinan and galactan) in the samples.

Santa Rosa samples, for extractable and polyoses obtained no significant differences between species. In this case, the species *A. melanoxylon* recorded the highest percentage consistently over time.

Theoretical yield of bioethanol

Analyzing the bioethanol yield in function of the surface is economically important because it is related with the amount of land required to supply a hypothetical biorefinery of a specific size that should be determined by the theoretical yield of the woody species established in the trials. Considering glucan as the most important carbohydrate for ethanol production, because glucose is highly fermentable for many microorganisms (XU et al., 2009), the average theoretical yield at 48 months in Llohué and Santa Rosa environments on the basis of both mass and surface is presented in Figures 2 and 3.

Wood and bioethanol yields per hectare for *A. melanoxylon* in Santa Rosa environment at the three assessed densities are low, between 2.5 and 3.5 $t \cdot ha^{-1}$, not exceeding the barrier of 1,500 L bioethanol in the months of the study (Figure 3). On the other hand, at Llohué environment, the yields of wood per hectare were above 9.5 t ha^{-1} for plantation densities of 5,000

TABLE 3 Content of extractable, lignin, glucan, polyoses, acetyl, and percentage of theoretical bioethanol of the biomass of species planted in Santa Rosa (sandy areas) from 2007 to 2011, calculated using 90% of efficiency in sugars to bioethanol conversion. Values represent the mean and the standard deviation of the sample.

Month	Species	Density	Percentage					% p/p theoretical bioethanol 90% efficiency
			Extractable	Total lignin	Glucan	Polyoses*	Acetyl	
11	*A. melanoxylon*	5,000	6.54 ±0.23	28.15 ±0.95	31.6 ±1.30	16.85 ±3.80	3.84 ±5.85	16.12 ±0.74
11	*A. melanoxylon*	7,500	0 ±0.00	0 ±0.00	0 ±0.00	0 ±0.00	0 ±0.00	0 ±0.00
11	*A. melanoxylon*	10,000	0 ±0.00	0 ±0.00	0 ±0.00	0 ±0.00	0 ±0.00	0 ±0.00
11	*E. globulus*	5,000	4.60 ±0.44	27.70 ±0.77	28.47 ±0.67	17.13 ±2.23	3.74 ±3.33	14.52 ±0.38
11	*E. globulus*	7,500	5.10 ±0.20	27.80 ±1.03	27.53 ±0.67	17.97 ±1.97	3.79 ±2.40	14.04 ±0.38
11	*E. globulus*	10,000	5.25 ±0.07	27.55 ±1.65	29.70 ±1.30	17.35 ±1.15	3.76 ±3.15	15.15 ±0.74
11	*E. nitens*	5,000	5.80 ±0.2	29.67 ±0.42	25.87 ±1.87	17.40 ±1.33	3.65 ±2.07	13.19 ±1.06
11	*E. nitens*	7,500	5.55 ±0.64	29.67 ±1.50	25.17 ±1.57	17.67 ±0.60	3.74 ±4.47	12.84 ±0.89
11	*E. nitens*	10,000	6.10 ±0.28	28.67 ±1.10	27.03 ±1.37	17.20 ±0.77	3.83 ±1.95	13.79 ±0.77
23	*A. melanoxylon*	5,000	4.45 ±0.21	22.13 ±1.27	38.17 ±1.00	23.07 ±0.87	3.50 ±1.10	19.47 ±0.57
23	*A. melanoxylon*	7,500	4.75 ±0.64	22.30 ±2.43	37.67 ±0.80	20.57 ±1.83	3.53 ±1.70	19.21 ±0.45
23	*A. melanoxylon*	10,000	3.95 ±1.06	21.80 ±2.23	42.87 ±1.43	22.03 ±1.83	3.50 ±1.63	21.86 ±0.81
23	*E. globulus*	5,000	3.90 ±0.36	26.00 ±1.47	35.70 ±0.83	23.03 ±2.47	4.07 ±3.27	18.21 ±0.47
23	*E. globulus*	7,500	5.33 ±0.67	26.10 ±1.77	31.80 ±1.90	20.83 ±1.87	3.97 ±1.70	16.22 ±1.08
23	*E. globulus*	10,000	3.21 ±0.23	26.87 ±1.30	32.93 ±1.40	19.90 ±3.10	4.20 ±1.43	16.80 ±0.79
23	*E. nitens*	5,000	3.40 ±0.46	27.10 ±1.40	37.23 ±0.40	25.03 ±2.47	4.13 ±1.60	18.99 ±0.23
23	*E. nitens*	7,500	3.37 ±0.59	26.37 ±1.43	35.90 ±0.47	22.50 ±1.57	4.10 ±1.33	18.31 ±0.26
23	*E. nitens*	10,000	5.20 ±0.99	26.43 ±1.77	32.70 ±0.83	22.10 ±2.10	4.30 ±3.30	16.68 ±0.47
36	*A. melanoxylon*	5,000	7.70 ±0.28	21.17 ±1.83	36.93 ±1.13	17.40 ±2.40	3.37 ±3.13	18.84 ±0.64
36	*A. melanoxylon*	7,500	6.25 ±0.64	21.67 ±2.43	37.20 ±0.57	18.23 ±2.52	3.50 ±1.03	18.97 ±0.32
36	*A. melanoxylon*	10,000	7.80 ±0.84	21.37 ±2.47	34.33 ±1.50	18.00 ±3.70	3.30 ±4.03	17.51 ±0.85
36	*E. globulus*	5,000	5.50 ±0.99	28.33 ±1.03	35.53 ±1.73	19.10 ±2.90	4.33 ±3.63	18.12 ±0.98
36	*E. globulus*	7,500	5.65 ±0.49	28.70 ±1.20	36.43 ±0.90	19.27 ±5.15	4.00 ±5.80	18.58 ±0.51
36	*E. globulus*	10,000	4.15 ±0.28	26.33 ±1.60	38.57 ±1.27	19.00 ±2.92	5.43 ±6.47	19.67 ±0.72
36	*E. nitens*	5,000	5.20 ±0.23	27.50 ±2.13	36.03 ±0.93	19.87 ±3.57	5.70 ±3.90	18.38 ±0.53
36	*E. nitens*	7,500	4.70 ±0.47	29.33 ±1.07	36.40 ±1.60	19.53 ±4.48	5.50 ±6.87	18.56 ±0.91
36	*E. nitens*	10,000	4.85 ±0.21	25.60 ±1.73	36.63 ±0.53	20.57 ±3.88	5.77 ±6.53	18.68 ±0.30
48	*A. melanoxylon*	5,000	6.70 ±0.38	22.89 ±0.81	39.53 ±1.23	19.12 ±1.15	3.64 ±1.80	20.16 ±0.70
48	*A. melanoxylon*	7,500	6.35 ±0.54	24.43 ±2.32	39.53 ±0.92	19.14 ±0.94	3.69 ±2.63	20.16 ±0.52
48	*A. melanoxylon*	10,000	5.80 ±0.68	22.97 ±1.47	39.62 ±3.48	18.94 ±0.69	3.63 ±1.75	20.20 ±1.97
48	*E. globulus*	5,000	5.45 ±0.65	28.44 ±1.34	34.90 ±1.89	17.45 ±1.87	3.63 ±3.07	17.80 ±1.07
48	*E. globulus*	7,500	5.85 ±0.78	29.23 ±0.80	35.30 ±0.88	17.64 ±1.05	3.77 ±3.11	18.00 ±0.50
48	*E. globulus*	10,000	5.15 ±0.56	28.74 ±1.44	36.01 ±0.28	18.39 ±0.84	3.91 ±0.60	18.36 ±0.16
48	*E. nitens*	5,000	5.44 ±0.33	29.56 ±1.84	35.21 ±0.71	18.36 ±0.80	4.13 ±2.12	17.96 ±0.40
48	*E. nitens*	7,500	4.89 ±0.39	28.01 ±1.71	35.26 ±0.96	17.70 ±1.59	3.90 ±1.46	17.98 ±0.54
48	*E. nitens*	10,000	4.44 ±0.35	29.40 ±0.59	34.28 ±2.58	18.15 ±2.08	3.95 ±3.24	17.48 ±1.46

* Represent the concentration of total polyoses from hemiceluloses (xylan, arabinan and galactan) in the samples.

TABLE 4 Variance analysis of extractable, total lignin, glucan, polyoses and acetyl yield (%) with repeated measurements.

Environment/effect	Number DF	Density DF	Pr > F				
			Extractable	Total lignin	Glucan	Polyoses	Acetyl
Llohué:							
Species	2	69	<0.0001	<0.0001	0.0206	<0.0001	<0.0001
Density	2	69	0.1457	0.1670	0.1630	0.3376	0.1685
Time	3	69	<0.0001	<0.0001	<0.0001	0.0485	<0.0001
Species × Density	4	69	0.5808	0.3988	0.2076	0.5010	0.3917
Species × Time	6	69	<0.0001	0.0723	0.0022	0.2099	0.0699
Density × Time	6	69	0.4603	0.3064	0.4343	0.6304	0.3155
Species × Density × Time	12	69	0.1812	0.8048	0.5687	0.6765	0.8134
Santa Rosa:							
Species	2	63	0.0839	<0.0001	<0.0001	0.1725	<0.0001
Density	2	63	0.4099	0.1066	0.3650	0.9179	0.3300
Time	3	63	<0.0001	<0.0001	<0.0001	<0.0001	0.0075
Species × Density	4	63	0.0099	0.9469	0.4828	0.8638	0.2866
Species × Time	6	63	<0.0001	0.0332	0.0005	0.0015	<0.0001
Density × Time	6	63	0.8308	0.4920	0.4018	0.1765	0.9777
Species × Density × Time	10	63	0.1264	0.6793	0.2797	0.7854	0.9535

Santa Rosa samples, for extractable and polyoses obtained no significant differences between species. In this case, the species A. melanoxylon recorded the highest percentage consistently over time.

and 10,000 trees per hectare and reaching a theoretical bioethanol between 3,500 and 5,400 L·ha^{-1}. In the case of the plantations at 7,500 trees·ha^{-1}, yields were t·ha^{-1} and the bioethanol values were close to 2,200 L·ha^{-1} (Figure 2).

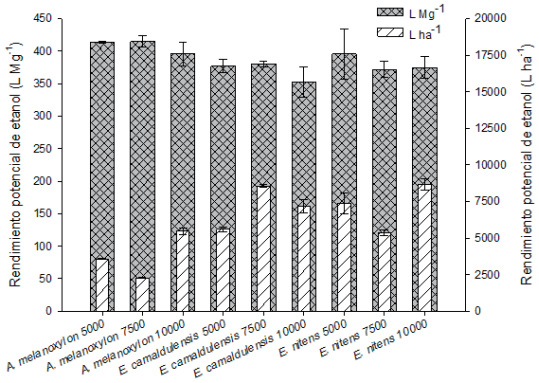

FIGURE 2 Bioethanol production potential in the rainfed (Llohué). Gray columns with grids represent the production in L·t^{-1}. White columns with diagonals represent L·ha^{-1}. Error bars represent standard deviation.

For *E. globulus* in Santa Rosa, the highest yields of wood per hectare (22 t·ha^{-1}) were obtained in plantations with 5,000 trees·ha^{-1}, coinciding with the highest yield of theoretical bioethanol for this species after 48 months, which was of 8,140 L·ha^{-1}. Plantations with 7,500 and 10,000 trees·ha^{-1} at month 48 showed wood yields between 11 and 18 t·ha^{-1}. On the other hand, bioethanol yields were 6,504 and 3,994 L·ha^{-1}, respectively (Figure 2).

Results indicate that for *E. nitens*, the highest yields were obtained in the plantations with 7,500 trees·ha^{-1} after 48 months, with yields of 35 t·ha^{-1} of wood at Santa Rosa and an average theoretical bioethanol yield close to 12,200 L ha^{-1} (Figure 2) in the last month of the assessment. In the same environment, plantations with 5,000 and 10,000 trees·ha^{-1} showed yields of 25 and 29 L·ha^{-1} respectively. In Llohué, the highest biomass yield was obtained by the density of 10,000 tress·ha^{-1}, with 23.2 t·ha^{-1} and bioethanol yields of 8,682 L·ha^{-1} (Figure 3). The species *E. camaldulensis* established at 7,500 plants·ha^{-1} in Llohué recorded wood yields of 22 t·ha^{-1} and a theoretical bioethanol yield of approximately 8,559 L·ha^{-1} after 48 months. In plantations with densities of 5,000 and 10,000 trees·ha^{-1}, the wood yields were 14 and 20 t·ha^{-1} respectively, equivalent to a theoretical bioethanol production of 5,603 and 7,171 L·ha^{-1}.

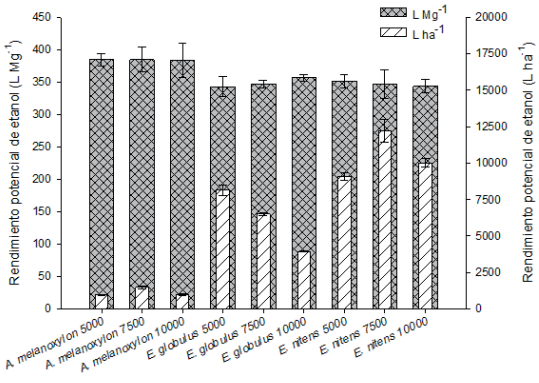

FIGURE 3 Bioethanol production potential in Sandy areas (Santa Rosa). Gray columns with grids represent the production in L·t^{-1}. White columns with diagonals represent L·ha^{-1}. Error bars represent standard deviation.

Summarizing, biomass production was a significant predictor on the ethanol yield per surface unit (Santa Rosa, F = 28,679.17, df = 1 and 107, P<0.001 y, Llohué, F = 8,501.96, df = 1 and 107, P<0.001). The adjusted R-square was 0.99 and 0.98 for Santa Rosa and Llohué respectively, for the relation between biomass production and ethanol yield per surface (Figure 4). The ethanol average conversion efficiency was 368.23 L·t^{-1} with a standard deviation of 40.75 in the two environments and in all studied months. Variation in the ethanol yield was entirely due to the variation in the biomass yield (Figure 4).

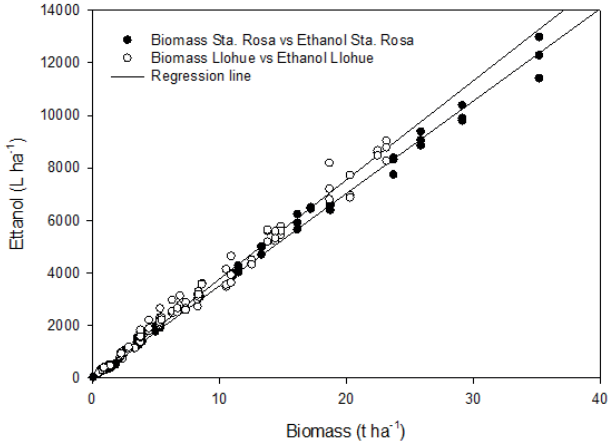

FIGURE 4 Correlation between ethanol yield (L·ha^{-1}) vs. biomass production (t·ha^{-1}). Black dots represent estimations for Santa Rosa and white dots, for Llohué.

DISCUSSION

The LCB has been proved to be one of the main renewable feedstocks for sustainable ethanol production in near future. LCB from SRWC is available in any season with low and stable price, rich in carbohydrates and non-competitive to food and feed. Extensive research efforts have been made in recent years on the technical aspects lignocellulosic ethanol production. Various integrations of individual technological steps have been investigated and few of them showed promising such as SSF, the principle of these approaches remains the same, rather fundamental steps should be followed for efficient conversion of cellulose and hemicellulose into ethanol (PAULOVA et al., 2015).

Both globally and nationally, there are great expectations on the yields of SRWC's with energy

purposes. In Chile, the State provides a subsidy through the Law Decree N° 701, as specified in the Table of general forestation costs for plantations with dendroenergetic purposes. To date, this subsidy is only granted for plantations of the genus *Eucalyptus* (CONAF, 2011).

Searle and Malins (2014) argue that the yields are reduced if they are produced at a semi-commercial scale and if the crops are planted in sub-optimal lands. In their revision, these authors recorded yields of 14-51 and 0-17 $t \cdot ha^{-1} \cdot year^{-1}$ for agricultural and marginal lands, respectively. In the sites in which this research was carried out -considered marginal because they only support subsistence farming- the best yields in dry biomass for *Acacia* (3.3 $t \cdot ha^{-1} \cdot year^{-1}$) and for *Eucalyptus* (11.1 $t \cdot ha^{-1} \cdot year^{-1}$) were achieved.

In the case of the chemical composition, this study it was found that the glucan - total carbohydrates ratio for *Eucalyptus* samples was 60.4%, whereas for *Acacia*, this ratio was 60.7%. Similar recovery of glucan from the solid fraction of *Eucalyptus* wood have been reported by Muñoz et al. (2011) and Monrroy et al. (2012), whereas for *Acacia*, the studies by Muñoz et al. (2007) and Yáñez et al. (2009) can be mentioned.

For the remaining elements studied, i.e. extractable, lignin, polyoses and acetyl. In *A. melanoxylon*, they are similar to those reported by López (2007), both average values and the range of variation. In eucalyptus, Lienqueo et al. (2016) obtained slightly higher polyoses content values than those recorded in this study, using the same saccharification and fermentation simultaneous procedure.

Regarding the outcomes in the average ethanol yield that reached 395.9 $L \cdot t^{-1}$ for *A. melanoxylon*, these values slightly lower than those reported by López (2007), who found bioethanol yields of 405.6 $L \cdot t^{-1}$, although at a much higher age range than those recorded in this study research (7-29 years). Records on the bioethanol yield for the genus *Acacia* have been reported by Muñoz et al. (2007) 379 $L \cdot t^{-1}$; by Yáñez et al. (2009) with 387 $L \cdot t^{-1}$ and by Ferreira et al. (2011) with 407.7 $L \cdot t^{-1}$ in *A. dealbata*; and by Ko et al. (2012) with 351.1 $L \cdot t^{-1}$ in *A. confusa*.

For *E. globulus* the ethanol average yield was 348.7 $L \cdot t^{-1}$. These values are found within the range of other studies, such as Muñoz et al. (2011) 381.0 $L \cdot t^{-1}$; Santos et al. (2011) 389.4 $L \cdot t^{-1}$; Ko et al. (2012) 333.5 $L \cdot t^{-1}$, Monrroy et al. (2012) 326.9 $L \cdot t^{-1}$ and Rodríguez-López et al. (2012) 335.3 $L \cdot t^{-1}$. In *E. camaldulensis* and *E. nitens,* yields of 370.1 and 363.9 $L \cdot t^{-1}$ were found, respectively. Nevertheless, the records on ethanol yield are scarce in literature. Among them, ZHENG et al. (2007) obtained 361.7 $L \cdot t^{-1}$ in *E. camaldulensis,* and Santos et al. (2011) achieve 380.4 $L \cdot t^{-1}$ in *E. nitens*. These records are close to those found in this study.

Zamora et al. (2014) in willow hybrids and native willow after a single 3-year harvest cycle on marginal lands in central Minnesota, found a biomass productivity of 5.34 $t \cdot ha^{-1} \cdot yr^{-1}$ with ethanol yield of 5913 $L \cdot ha^{-1}$ much lower than the one recorded in our study for *A. melanoxylon* 5344.7 (the least) and 15482.3 for *E. globulus* (the highest).

However, because of the results obtained, it is imperative to take into account that the chemical composition of the biomass is normally affected by temperature and humidity of the soil (ADLER et al., 2006; ADLER et al., 2009). In the present study, the lowest glucan contents were observed in the species *A. melanoxylon*, *E. globulus* and *E. nitens* harvested in Santa Rosa. The strong interaction between treatment and environment itself generally exceed the species – treatment effect. Thus, if the investors of SRWC's are in the quest of raw material for ethanol production, they will maximize their revenue by means of the identification of species with high biomass yield and not necessarily in function of the ethanol theoretical potential of the species.

CONCLUSIONS

Due to their chemical composition and a polysaccharide-yielding potential, biomass of SRWC's can be used as feedstock for second-generation bioethanol. However, the chemical composition of biomass and glucan-yielding potential has been shown to vary between the species and time (rotation period).

Compared with the other species, *Eucalyptus* and *Acacia* biomass were characterized by a high content of glucan and polyoses and a lower content of lignin. It has been shown that *E. nitens* at 10,000 $tree \cdot ha^{-1}$ can yield 2,171 L of ethanol $ha^{-1} \cdot year^{-1}$ at Llohue, medium fertility soil. A higher yield of ethanol (around 42%), was obtained in *E. nitens* at 10,000 $tree \cdot ha^{-1}$ with yield of 3,054 L of ethanol $ha^{-1} \cdot year^{-1}$ at Santa Rosa, which is a low fertility soil.

This study has shown that the choice of SRWC species used as a source of polysaccharides must take into account the species, planting density, harvest cycle and site. It must be carefully selected to ensure high biomass yield per unit area, because differences in the potential yield of glucan and polyoses per 1 ha are of up to 200%.

ACKNOWLEDGMENTS

This work was supported by the Fondo de Innovación Tecnológica de la región del Biobío, INNOVA Bio-Bio, project N° 06 PC S1-33 "Desarrollo de protocolos para la producción de biomasa de especies forestales de rápido crecimiento y corta rotación para la generación de bioenergía".

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